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Short title: **Rumination as diseases predictor**

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Summary

Aim of this Research Paper was to study the changes in rumination time (RT) in the days before the recording of diseases (specifically: mastitis, reproductive system diseases, locomotor system issues, and gastroenteric diseases),and to give the basis for a possible future use of this novel trait as a predictive tool for herd management. We built predictive models to assess the association between RT and diseases, using the former as the outcome variable, and to study the effects of the latter on the former. Those models, if properly working, could be used, after further validations, as tools able to identify a disease category before the occurrence of the clinical symptoms. The average Pseudo-R^2 of the fitted models was moderate to low, and this could be due to the fact that RT is influenced by other additional factors which have a greater effect than the predictors used here. Although remaining in a moderate-to-low range, the average Pseudo-R^2 of the models regarding locomotion issues and gastroenteric diseases was higher than the others, suggesting the greater effect of these diseases on RT, especially, as expected, of the gastroenteric ones.

Key words: rumination, animal welfare, dairy cow, predictive model

Over the last years, animal welfare has become a major research area in animal husbandry. This increasing interest has been driven by both ethical concerns and the increasing attention of consumers on product quality rather than quantity (Thornton, 2010). One of the most accepted definition of the animal welfare concept are the “five freedoms”, formalised in July 1979 in a report by the Farm Animal Welfare Advisory Committee. A review by Webster (2001) is available, where these “freedoms” are listed and explained. Nevertheless, being a multidimensional factor (Fraser, 1995), animal welfare assessment is not straightforward and it is dependent on different human cultures, traditions and religious faiths (Szűcs et al. 2012). Rumination is described as the process of regurgitation, re-mastication, salivation, and swallowing of feed to reduce the particle size and enhance fibre digestion(Erina et al. 2013). Rumination Time (RT, i.e., the number of minutes spent by a cow during a determined time interval) has been associated with rumen welfare, since it increases the production of saliva, which act as a buffer for the ruminal pH (Beauchemin, 1991).

The development in early 2000s of automatic systems able to record and store a large amount of different parameters related to milk yield and cow activity, among others, increased the possibility to investigate changes in RT, and in its relationships with those above-mentioned parameters. Some studies have shown that a RT decrease might be an indicator of unfavourable psychological (acute stress: Herskin et al. 2004; anxiety: Bristow & Holmes, 2007) and pathological (hypocalcaemia: Hansen et al. 2003) conditions. More recently, RT has been further investigated to assess its relationship with the physiological changes linked with calving and estrus events. Clark et al.(2015) correlated RT and activity time, concluding that there was a distinct decline in the duration of rumination pre-partum, which could be successfully used to predict the cows’ day of calving. Dolecheck et al.(2015) described the estrus-related changes in parameters automatically recorded by different commercial systems and assessed the potential use of this data collecting technology for estrus detection. The relationship between RT and diseases has not been fully investigated yet. Some recent

studies showed that common dairy farm diseases (Van Hertem et al. 2013; Liboreiro et al. 2015; Talukder et al. 2015) significantly decrease the RT. Stangaferro et al. (2016a, b, c) demonstrated that metabolic and digestive disorders, mastitis, and metritis have a negative effect on RT and could be predicted by analysing patterns in RT changes.

The hypothesis tested in this study is that, by predictive modelling, a trait recorded by automatic systems (e.g., RT) could be used as predictive tools for incoming diseases. Furthermore, aim of this study was also to describe changes in RT in the days before the recording of different diseases.

Material& Methods

Data collection

The animals monitored in this study were 259 Italian Holstein cows reared in a commercial farm located in Mantua province, Lombardy (Northern Italy). All the animals were fed total mixed ration (TMR), milked twice a day and grouped in pens (lactating, pre-calving, and infirmary). RT data were recorded using the Heatime HR system (SCR Engineers Ltd., Netanya, Israel) from the 24th of September 2014 to the 6th of October 2015, for a total of 377 consecutive days. This system is composed by a neck collar with a tag containing a microphone to monitor rumination and an accelerometer to quantify activity (as validated by Schirmann et al. 2009). The raw data are then processed and summarised as 2-h intervals by the herd management software DataFlow II (SCR Engineers Ltd.), where all the information regarding each single animal (e.g., ID number, age, parity) is recorded, and then downloaded in a spreadsheet file.

The list of diseases was obtained from the farm management software, where they were recorded soon after the diagnosis of the veterinary, both during routine or requested visits to the farm. Their incidence is reported in Table 1.All of the recorded diseases were used in this analysis, regardless of their known effect or association with RT changes. Excluding mastitis, other diseases recorded in the software were

grouped into three main classes, according to a veterinary classification: reproductive system diseases (i.e., metritis, retained foetal membranes, and ovarian cysts), locomotor system issues (i.e., lameness and generic leg infections), and gastroenteric diseases (i.e., abomasal displacement and dysentery). Other than the disease presence, no other information was available (e.g., no specific details on which type of mastitis or infection was diagnosed). In order to create a case-control dataset, for each disease, only the cows that manifested a disease at least once were kept in the dataset, hence removing all the animals that did not experience any disease during the study. Furthermore, all of the diseases were then summarized in a "generic disease" variable, which described with 1/0 (i.e., presence/absence, respectively) the occurrence of at least one sanitary event.

Statistical analysis

This study was composed of two main parts: in the first one, mixed models were used to analyse the effects of diseases on 2-h rumination time. All the models were fitted using the lme4 package (Bates et al. 2015) in R (version 3.2.5; R Foundation for Statistical Computing, Vienna, Austria). Subsequently, the statistical significance of the model was checked with the lmerTest package (Kuznetsova et al. 2015). Model 1 was fitted with the general disease variable:

Model 1) $$rum\_mean_{ijk} = generic\_disease_i + date_j + animal_k + \varepsilon_{ijk},$$

where rum_mean_{ijk} is the mean rumination time for animal k affected by a generic disease in test-day j; $generic\_disease_i$ is the presence or absence of an unhealthy status; $animal_k$ is the random effect of the k^{th} animal; $date_j$ is the random effect of the j^{th} test day; and ε_{ijk} is the random residual effect.

Model 2 was fitted including as independent variables each disease category:

Model 2) $$rum\_mean_{ijklmn} = reprod_i + mast_j + locom_k + gastroent_l + animal_m + date_n + \varepsilon_{ijklmn},$$

where rum_mean_{ijklmn} is the mean rumination time for the animal *m* in the test-day *n*, affected or not by *reprod i*, *mast j*, *locom k*, and *gastroent l*; $animal_m$ and $date_n$ are the random effects; and ε_{ijklmn} is the random residual effect.

In the second part of this study, a sliding windows approach was applied to the data to investigate the change in rumination time in a total of six different windows before and after the disease event (i.e., generic disease, reproductive diseases, mastitis, locomotor system issues, and gastroenteric diseases): the windows dimensions were of 1, 3, and 5 days, symmetrically set around the disease event. This approach is widely used in genomic analyses (e.g., linkage disequilibrium and signatures of selection identification), but is seldom applied outside of this field. On each window, the 2-h rumination mean, standard deviation (SD), and slope (from a linear regression of the rumination on the days in the window) were calculated. Furthermore, for each of these new parameters, summary statistics (i.e., mean ± SD) were calculated. Four different generalized linear models (Logistic regression) were then fitted on the window before the sanitary record, each with the disease event as a binary response (i.e., presence/absence: 1/0) and the afore-mentioned calculated parameters as predictors (Models 3.a to 3.d):

3.a) $disease = rum\_mean$

3.b) $disease = rum\_sd$

3.c) $disease = rum\_slope$

3.d) $disease = rum\_mean + rum\_sd + rum\_slope$

where *disease* is the presence or absence of one of the five cases analysed; *rum_mean* is the averaged rumination time in the window; *rum_sd* is the standard deviation of the rumination in the window; and *rum_slope* is the coefficient from the regression of the RT on the days in the window. AIC (Akaike information criterion) and McFadden's Pseudo R^2(McFadden, 1974) were calculated to compare the models and assess which predictors and which window best fitted the data.

Results

Single Variable Comparison

The mean (±SD) RT of the animals in the herd, throughout the whole 377 days, was 46.99±11.07 min/2h. The effect of the disease presence on the 2-h RT was significant in every analysed case ($p=0.001$ and $p<0.001$, locomotor issues and all the other cases, respectively). Gastroenteric diseases had the largest effect, lowering RT by 9.91 min/2h, while reproductive ones had the smallest, 1.08 min/2h. Only three cows suffered from gastroenteric diseases, therefore, even if highly significant, the result must be interpreted with caution. The differences between the means (in min/2h), the number of cows analysed, the ratio between positive and negative cases (case-control ratio), and the p-value from at-test, performed to assess if the differences between the two statuses were significant, are reported in Supplementary Materials, Table S1.

Multiple Variables Comparison.

The fixed effects of Model 1 (estimated values, SEM and p-values) are reported in Table 2, and their analysis of variance is reported in Table 3a. The random effects variances and standard deviations are reported in Table 3b. In this model, the diseased status had a significant effect ($p<0.001$) on RT, lowering it by 2.22 min/2h. The inclusion of the effect of the parity as predictor in the models was considered. However, with the inclusion of this effect in a preliminary test, the resulting model had worst fit on our data (probably because this effect was confounded with the animal and date random effects), and parity was therefore removed.

The variance explained by the animal effect was the 12.33% of total variance explained, and it was 6.30 times larger than the day effect variance. McFadden's Pseudo-R^2 of the model was 14.8%.

The fixed effects of Model 2 (estimated values, SEM and p-values are reported in Table 2) were statistically tested in the same way of Model 1, and the results are summarized in Table 3a, while the

155 random effects variances and standard deviations are reported in Table 3b. The featured diseases in this
156 model negatively affected RT, with a RT decrease which ranged from -1.73 to -5.76 min/2h
157 (reproductive and gastroenteric diseases, respectively).Similarly to the results of the general disease
158 model, the variance explained by the animal effect (12.27% of the total variance explained) was larger
159 than the date effect (6.25 times larger). Pseudo-R^2 of the model was 14.8%. Least Square Means (LSM)
160 of 2-h RT by the different diseases are reported in Figure 1. LSM were calculated for each single
161 couple of diseased/non-diseased animals and were, therefore, different for different classifications.
162 Variance inflation factor (VIF) was used as a diagnostic for multicollinearity within the models. In both
163 of the models, multicollinearity was negligible. ANOVA was subsequently performed to assess which
164 model (general disease variable vs grouped diseases) better fitted the data: the two models were
165 statistically different ($p<0.001$).
166
167 *Sliding windows analysis*
168 Regarding the sliding window analysis, summary statistics of the three disease predictors related to RT
169 (i.e., mean, SD, and slope), for both the window before and after the disease event, as shown in
170 Supplementary Materials, TableS2.In order to investigate if the pathological event changed the
171 observed parameters “before” and “after” event occurrence, a t-test with a threshold of 0.05 for the p-
172 value was used.For the generic disease analysis, only the slope was statistically different from before to
173 after the event (different in all the three window’s sizes).In the reproductive system diseases analysis,
174 significant differences were identified only in the slope for windows’ sizes of 3 and 5 days. In the
175 mastitis analysis, the 1day window mean and all the slopes were statistically different. In the locomotor
176 system issues analysis, the 5days window mean and all the slopes excluding the one in 1day window
177 were statistically different. Lastly, in the gastroenteric diseases, a similar pattern as in the locomotor

system issues were observed, i.e., all the slopes excluding the one in 1day window were statistically different.

Logistic models were also fitted to the data: the estimate of the β, the odds ratio for the disease presence, the AIC of the model, and its Pseudo-$R^2$are reported in Supplementary Materials, Table S3.In all of the five cases, the best model (i.e., lower AIC and higher Pseudo-R^2) was always Model 3.d, which fitted all the three considered predictors. For the generic disease analysis, mean, SD, and slope models showed significant effects in models from 3.a to 3.c (with a maximum Pseudo-R^2 of 2.99%, 0.95%, and 6.02%, respectively), with the only exception of the 1day window SD models, in which the effect is not significant. In Model 3.d, SD was never significant. Nevertheless, this model had the highest Pseudo-R^2 and the lowest AIC for all the three windows' sizes. The reproductive system diseases model analysis showed a similar situation of the general disease analysis, although with lower Pseudo-R^2 values. Another important difference was the complete non-significance of all the models using SD as a predictor (Model 3.b). The mastitis model analysis had a similar pattern as the general disease one: the only non-significant window's size in the single-predictor models (i.e., Model 3.a to 3.c) was the Model 3.b, window's size of 1 day (SD). The maximum Pseudo-R^2 were 1.50%, 1.14%, and 4.10%, respectively. Regarding Model 3.d, with window's size of one, SD was not significantly effective on RT. The locomotor system issues analysis showed a different pattern from the previous ones: the mean RT model (Model 3.a) had Pseudo-R^2 ten-fold higher than generic disease, reproductive diseases and mastitis ones. Similarly to the reproductive diseases analysis, however, Model 3.b was never statistically significant. Lastly, the gastroenteric diseases model analysis had, on average, the highest Pseudo-R^2 of all the analyses. The only non-significant window's size was the 1 day slope window. In the models with the three predictors (i.e., Model 3.d), both SD and slope were never statistically significant. Nevertheless, the Pseudo-R^2 ranged from 43.89% to 58.81%.

The best models for each case, selected using the AIC and the Pseudo-R^2 as criteria, were always Model 3.d, but with a window's size of 5 days for the generic disease (Pseudo-R^2=6.47%), a window's size of 3 days for reproductive diseases (Pseudo-R^2=7.16%), a window's size of 1 day for mastitis (Pseudo-R^2=5.61%), a window's size of 5 days for locomotor issues (Pseudo-R^2=16.17%), and a window's size of 1 day for gastroenteric diseases (Pseudo-R^2=58.81%).

Discussion

Automated rumination and activity monitoring could be used to identify diseases earlier than through clinical diagnosis performed by trained personnel. This confirms the results obtained by Stangaferro et al. (2016a, b, c). All the comparisons between mean RT of diseased and non-diseased animals resulted statistically significant. Nonetheless, the differences were small and this type of comparison does not account for any interaction between variables and, therefore, it can identify large effects only. However, all of the different diseases' effects were confirmed as statistically significant in the multiple variable approach too. The difference between the effects in the single and in the multiple variable comparison comes from the effect of the cow and the test-day, taken into account as random effects using the mixed model in the latter. These random effects should reduce the bias due to the correlation between the repeated measures.

The variance explained by the animal effect was larger than the date effect variance in both cases (i.e., general and grouped disease), suggesting that the observed variability is mainly due to the animal effect rather than to the test-day. This result is in accordance with the one from Byskovet al. (2015), where the authors observed that the 48% of the total variation in RT was due to the animal effect, whereas feed intake accounted for the 32%.

223 Compared through ANOVA, the two models were statistically different. Specifically, the model
224 including specific variables for each disease fits the data better. Using mixed models, the effect of
225 diseases on RT was confirmed in this study, though the model could be improved by adding further
226 predictors (e.g., feed intake and diet of the animals, which was not available for this
227 experiment).Results obtained with mixed models are in accordance with, and further expand, the results
228 by Stangaferro et al. (2016a, b, c).
229 With the sliding windows approach, we wanted to test if different features of RT in the days before a
230 disease diagnosis could be predictive of the disease itself. This predictability would be desirable, since
231 the detection of a disease as early as possible allows for a more immediate sanitary intervention. The
232 features selected were the mean, the SD, and the regression slope of RT on time to disease. A
233 difference in the slope before and after the diagnosis means that the rumination changes its trend
234 (negative or positive). In the significant cases, the "after" windows showed a positive (or less negative)
235 trend, while the "before" window had always a negative one: this results could suggest that these
236 diseases affect rumination time, lowering it, and, since in our data the recorded event corresponds to
237 the vet visit and the treatment beginning, we saw the improving of the rumination in the "after"
238 window due to medical treatment. From a descriptive point of view, SD of the RT was never
239 statistically different before and after the disease event, while the mean showed a difference in the day
240 before mastitis only. Of the selected feature, then, only the slope should be considered as a predictor,
241 though the best model was always the one using all of the three features. The benefits from including
242 mean and SD in the model are larger than the disadvantages, and this is probably due to a better
243 representation of the phenomenon.
244 Different diseases could be predicted using different window size. Specifically, mastitis and
245 gastroenteric diseases are better described by the models using one single day before the clinical
246 diagnosis, while reproductive diseases and locomotor issues by the ones using 3 and 5 days,

247 respectively. The Pseudo-R^2 of the reproductive diseases and mastitis predictive models was low. This
248 could be due to the moderate ability to identify mild cases of metritis (Stangaferro et al. 2016c) and
249 mastitis caused by pathogens other than *Escherichia coli* through rumination changes. As stated by
250 Stangaferro et al. (2016b), intramammary infections caused by *E. coli* are more easily identified
251 because they are characterized by a severe inflammatory response, including sudden shock, sepsis, and
252 often death. On the other hand, even with a small number of animals with gastroenteric disease, models
253 predictive for these diseases had the highest Pseudo-R^2, in accordance with the high sensitivity detected
254 by Stangaferro et al. 2016a. Locomotor issues, which were not analysed in the above-mentioned
255 studies, showed a Pseudo-R^2in between the other cases.
256 The difference in the window size in each different disease could be due not only to the higher or lesser
257 effect of each disease on RT, but also to the different reaction time of the farmer in response to the
258 different symptoms detected on his animals. Different diseases are perceived differently by farmers
259 (e.g., mastitis is, from a commercial point of view, more fearful than other diseases) and, therefore,
260 they could require vet intervention with different urgency. Moreover, in order to obtain accurate
261 estimates at enough distance from the event it is necessary for the farmer to carefully consider the
262 occurrence of the events.

263

264 **Conclusion**

265 In this study we observed that common farm diseases (i.e., reproductive diseases, mastitis, locomotor
266 system issues, and gastroenteric diseases) significantly affect the 2-h interval RT, lowering it in
267 comparison to the one of healthy animals. Further studies should focus on exploring the possibility to
268 predict the onset of diseases using, as predictors, these novel phenotypes which we demonstrated
269 having a correlation with them. The growing presence of automatic recording systems, even in
270 medium-small farms, will allow researchers to have larger datasets to work with.

The authors thank SCR Engineers Ltd. (Hadarim, Netanya, Israel) for supplying the technologies used in this study, Milkline Srl (Gariga di Podenzano, Piacenza, Italy) for the technical support and "Bulgarelli Giacomo e Astore" dairy farm for the herd rumination raw data and health status recordings.

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Table 1. Recorded diseases and relative incidence in the data, in descending order.

Disease	Incidence
Generic diseases	57.14 %
Reproductive diseases	34.36 %
Mastitis	32.43 %
Locomotor issues	8.11 %
Gastroenteric diseases	1.16 %

Table 2. Models 1 and 2, 2-h interval. Fixed effects estimates, standard error of the means and significance by p-value. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < .< 0.1 < ns.

Fixed effects	Estimate	SEM	p-value	Significance
Model 1				
Intercept	47.46	1.63	< 0.001	***
generic_disease	-2.22	0.15	< 0.001	***
Model 2				
Intercept	47.41	0.26	< 0.001	***
reprod	-1.73	0.17	< 0.001	***
mast	-4.07	0.33	< 0.001	***
locom	-1.76	0.65	0.007	**
gastroent	-5.76	0.95	< 0.001	***

Table 3. Models 1 and 2, 2-h interval. **a)** Statistical test for fixed effects. The significance codes are: 0 < *** < 0.001 < ** < 0.01 < * < 0.05 < .< 0.1 < ns. **b)** Table of random effects, with their variances and standard deviations.

a)

Fixed effects	Sum Sq	Mean Sq	df	F-value	p-value (> F)	Significance
Model 1						
generic_disease	23387	23387	1	223.22	< 0.001	***
Model 2						
reprod	11305.8	11305.8	1	107.969	< 0.001	***
mast	15909.8	15909.8	1	151.938	< 0.001	***
locom	761.5	761.5	1	7.273	0.007	**
gastroent	3862.2	3862.2	1	36.883	< 0.001	***

b)

Random effects	Variance	Std. Dev.
Model 1		
$animal_i$	15.065	3.881
$date_j$	2.392	1.547
residual	104.772	10.236
Model 2		
$animal_i$	14.983	3.871
$date_j$	2.396	1.584
residual	104.713	10.233

Figure legends:

Figure 1:

LSM of Rumination Time (RT) by specific disease presence (i.e., “Diseased”) or absence (i.e., “Non-diseased”), calculated for each single couple of diseased/non-diseased animals. The number of diseased animals for each disease is 126 (generic diseases), 66 (reproductive diseases), 68 (mastitis), 16 (locomotor issues), and 3 (gastroenteric diseases).

341 **Figure 1:**

342